The Protective Effects of Simvastatin on Muscle in a Rat Model of Chronic Rotator Cuff Injury

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Introduction: Massive tears of the rotator cuff represent one of the most common pathologies that cause shoulder pain and discomfort, and this injury can limit function and negatively impact quality of life. Patients with chronic rotator cuff tears often suffer from muscle fiber atrophy and shortening, a decrease in strength, fibrosis of the extracellular matrix (ECM) and fat accumulation throughout the muscle, commonly referred to as fatty degeneration. Even with surgical repair of these chronic tears, almost half of patients do not experience improvement of their fatty degeneration 1 year post-surgery (1). The lipid that accumulates in torn rotator cuff muscles is an appealing therapeutic target for preventing inflammation and atrophy, as large lipid droplets often serve as sites of production for proinflammatory signaling molecules and cytokines (2). As cholesterol and other lipid derived molecules can function as potent inducers of inflammation in other diseases (2), ectopic lipid within torn rotator cuff muscles may serve the same purpose. Lipid lowering medications, such as statins (HMG CoA reductase inhibitors) are commonly prescribed to patients with hypercholesterolemia or cardiovascular disorders. There is a positive correlation between hypercholesterolemia and developing a rotator cuff tear (3), but despite the potential role that cholesterol plays in promoting local tissue inflammation, it is unknown whether statin medication could be used to decrease fatty degeneration in patients with torn rotator cuff muscles. We hypothesized that administering a statin medication, simvastatin, would protect torn rotator cuff muscles from developing fatty degeneration. To test this hypothesis we treated rats that underwent a full-thickness supraspinatus tear with simvastatin or vehicle for four weeks, and then removed the muscles to evaluate histological and biochemical markers of fat accumulation, inflammation and regeneration.

Methods: This study was approved by our IACUC. Male Sprague-Dawley retired breeders were placed in 2 groups, vehicle control (N=8 rats) and simvastatin treatment (N=8 rats). Each group underwent a supraspinatus tenectomy to simulate a chronically torn rotator cuff. Rats received once daily oral gavage of 20 mg/kg of simvastatin suspended in vehicle (1% HPMC), or vehicle alone, for 4 weeks following the tenectomy. At the end of the 4 weeks, muscles were harvested and prepared for muscle fiber contractility testing, histology and gene expression. The contractility of muscle fibers of 8-10 fibers per muscle were analyzed using standard techniques. For histology, muscles were stained with Oil Red O (ORO) and hematoxylin in order to visualize the fat present in the muscle tissue. The portion of the muscle that was used for gene expression was minced and total RNA was isolated using a miRNeasy kit (Qiagen). The RNA was reverse transcribed to cDNA using the RT2 First strand kit (Qiagen) and amplified in a CFX96 real time thermal cycler (Bio-Rad) using a custom array of primers for specific mRNA species and an RT2 SYBR Green qPCR mix (Qiagen). Expression of the mRNA transcripts was normalized to the stable housekeeping gene β-2 microglobulin (B2M). Differences between groups were tested using unpaired t-tests (P<0.05).

Figure 1. Representative histology showing a gross decrease in the accumulation of lipid simvastatin treated muscle (B) when compared to the control muscle (A).

Figure 2. Muscle fiber specific force (A) and gene expression comparing control and simvastatin treated rats (B). Differences between groups were tested using t-tests; *,
Results: Compared to control rats (Figure 2A), there was an increase in muscle fiber specific force production (sFo). For histology (Figure 1), there was a marked decrease in the amount of total lipid visible within and around the muscle fibers in the supraspinatus of the rats that received simvastatin. Using gene expression measurements for transcripts involved with ECM fibrosis and maintenance (Figure 2B), simvastatin treatment decreased the expression of type I collagen (Col1a2), fibroblast specific protein 1 (FSP-1) platelet derived growth factor receptor-α (PDGFRα), tenomodulin (Tnmd), matrix metalloproteinase (MMP)-2, MMP-14, and tissue inhibitor of matrix metalloproteinase (TIMP)-1 and -2. For markers of fat accumulation and muscle regeneration (Figure 2), there was a decrease in the expression of peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT-enhancer-binding proteins-α (C/EBPα), and embryonic myosin heavy chain (eMHC).

Discussion: Understanding the etiology of fatty degeneration is critical in order to improve the functional outcomes of rotator cuff repair surgery. This is the first study that examined the effect of a statin drug on the development of fatty degeneration in a rat model of chronic rotator cuff disease. Compared with the vehicle treatment, administration of simvastatin resulted in a clear decrease in the amount of fat accumulation that occurred after tear. The overall reduction in adipogenic and ECM genes, along with a decrease in eMHC expression and an increase in muscle fiber force production suggests that simvastatin treatment resulted in healthier muscle tissue after rotator cuff tear. Future studies will determine whether simvastatin acts by preventing inflammation or enhancing regeneration after rotator cuff tear.

Significance: This study demonstrates that simvastatin administration can enhance muscle function in rats that received a rotator cuff tear. Future studies that evaluate the ability of simvastatin to improve chronically torn rotator cuff muscles that undergo repair, as well as studies in patients, would provide further insight into the ability of statin medication to enhance clinical outcomes for patients with chronic rotator cuff tears.

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